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Research Note

Early *in vitro* selection of eutypine-tolerant plantlets. Application to screening of *Vitis vinifera* cv. Ugni blanc somaclones

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Eutypiosis, due to the ascomycete fungus *Eutypa lata* (Carter and Price 1973; Bolay and Moller 1977), affects a number of vineyards throughout the world. Several quality cultivars are sensitive such as Cabernet Sauvignon and Ugni blanc. In the Cognac region where Ugni blanc is the only variety grown, this disease is causing an important economic problem (Le Gall 1992).

The pathogenic agent, present only in the trunk and arms, acts from a distance causing symptoms in herbaceous stems, and can bring about the death of the grape stock. *Eutypa lata* synthesizes a compound that has been isolated and chemically characterized, hydroxy-4(methyl-3-butene-3 ynyl-1)-3 benzaldehyde, named eutypine (Tey-Rulh *et al.* 1991). It has been demonstrated that eutypine is a toxin which participates in the pathogenic action of *Eutypa lata*. Since there are no chemical pesticides to fight against eutypiosis, the selection of genetic resistance is essential. Owing to legal restrictions on grapevine cultivation in "appellation" areas, new interspecific hybrids cannot be grown. Biotechnological methods seem to be the only possible strategy for selection using two approaches. One is based on the appearance of spontaneous variants or toxin-induced variants (Fallot *et al.* 1990) and the other on genetic transformation.

The success of Ugni blanc regeneration by somatic embryogenesis allows the use of these methods of selection. Here, the first approach has been carried out, disease resistance being induced using toxin as a selection pressure agent to orient variability. This approach is successful for the selection of plants tolerant to parasitic fungi (Daub 1986). However, the tolerance of the plantlets obtained must be determined early *in vitro*.

The purpose of this research was to develop an early physiological *in vitro* test for screening tolerant clones and somaclonal variants of Ugni blanc with respect of eutypine. A bioassay has already been described, based on the confrontation of grapevine protoplasts with eutypine, enabling the discrimination between tolerant and very sensitive varieties of *Vitis vinifera* (Mauro *et al.* 1988). The new test is based on the revelation of the ability of plantlets to grow in the presence of eutypine.

The *in vitro* multiplication by micropropagation of several clones of Ugni blanc was achieved on hormone-free half-strength Murashige and Skoog's (1962) medium (MS/2 medium) supplemented with sucrose (20 g/l) and agar (7 g/l). To estimate the sensitivity of clones towards eutypine, 6 microcuttings were cultured on 60 ml of MS/2 medium in a Magenta™ vessel in the presence of 200 µM chemically synthesized eutypine (Defranq *et al.* 1993). This eutypine concentration appears to be well adapted for the discrimination between clones. Indeed, preliminary studies showed that higher concentrations of toxin totally inhibited the growth of most Ugni blanc clones.

After 6 weeks in culture, different parameters were studied. The data presented in the figure clearly show that the growth of clone B was dramatically affected by toxin whereas the development of plantlets of clone A was only slightly reduced in the presence of eutypine. These observations suggest that the bioassay offers the possibility of discrimination between clones inside the variety Ugni blanc.

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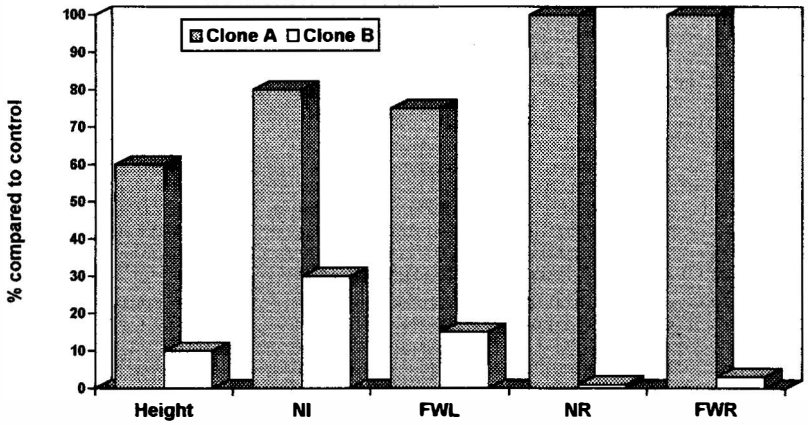

Figure: Effect of 200 µM eutypine on the growth and development of *in vitro* plantlets of two Ugni blanc clones, after 5–6 subcultures. Values represent, for each parameter, the mean for 12 plantlets. (Height: height of stem, NI: number of internodes, FWL: fresh weight of leaves, NR: number of roots, FWR: fresh weight of roots).

This *in vitro* test is now being used to select somaclones of Ugni blanc obtained from flower tissues via somatic embryogenesis with the method of MAURO *et al.* (1986) slightly modified. To date, several somaclones have been produced and they are now being tested. Some somaclones have been acclimatized, grafted and will be planted in the Charentes vineyard to test their ampelographic conformity, their technological suitability and their reaction to *Eutypa lata*.

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